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THE DESIRABILITY OF PHOSPHATES AS AN  
ADDITION TO CULTURE MEDIA FOR  
TUBERCLE BACILLI.

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# THE DESIRABILITY OF PHOSPHATES AS AN ADDITION TO CULTURE MEDIA FOR TUBERCLE BACILLI.

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During the course of a general chemical examination of tubercle bacilli of human origin, the writer, in collaboration with Dr. E. A. de Schweinitz, made an analysis of the ash of those organisms.<sup>a</sup> The medium upon which the bacilli were grown consisted of ordinary peptonized bouillon containing one-half of 1 per cent sodium chloride and 7 per cent glycerine, the bouillon being neutral at the time of inoculation. The culture flasks were kept in the thermostat at 37.5° C. for several weeks, or until a heavy growth had been obtained, when they were sterilized at 110° C. and the bacilli separated by filtration. After being washed thoroughly with distilled water, the bacilli were transferred to watch glasses and dried over sulphuric acid. The dried organisms were then finely powdered and thoroughly extracted with absolute ether and alcohol. They were then ignited at a low red heat until practically free of carbon, the heat being kept low enough at all times to avoid fusing the residue. The analysis of the ash obtained in this way gave the following results:

	Per cent.
Na <sub>2</sub> O .....	13.62
K <sub>2</sub> O .....	6.35
CaO .....	12.64
MgO .....	11.55
C and Si .....	0.57
P <sub>2</sub> O <sub>5</sub> .....	55.23

The most notable fact in this analysis was the absence of sulphates and chlorides and the presence of such large quantities of P<sub>2</sub>O<sub>5</sub>. If our present ideas regarding the constitution of proteids are correct, sulphur must have been present in at least small quantities in the bodies of the bacteria, and its absence in the ash can be explained by the supposition that it existed in organic combination only and was lost during the combustion. The large percentage of phosphoric acid indicated the desirability, if not the necessity, of the addition of some salt of phosphorus to culture media for tubercle bacilli. It was decided, therefore, to prepare a broth in which a phosphate should replace the

<sup>a</sup>Journ. Amer. Chem. Soc., Easton, V. XX, No. 8, p. 618. Aug., 1898.

chlorides and to compare such a broth with the ordinary sodium chloride bouillon which had been in use in this laboratory for a number of years and which is probably even now employed in most laboratories as a mineral addition to media for the cultivation of tubercle bacilli. The phosphate bouillon was prepared in a number of different ways. In some instances sodium ammonium phosphate was used, in others basic sodium phosphate, and in others acid potassium phosphate. These various lots of bouillon containing phosphate were compared with broth to which sodium chloride had been added in the usual proportions, all other conditions, such as the source of the beef extract, the percentage of glycerine, and the reaction of the media, being identical in the several lots of bouillon. It was found after the first inoculation of these cultures that those lots of bouillon which contained a salt of phosphorus grew more rapidly than the flasks containing sodium chloride, and this notwithstanding the fact that the bacilli with which the flasks were inoculated had been grown for years upon bouillon in which sodium chloride was always present.

Lots of agar tubes were prepared; some containing acid potassium phosphate; others containing sodium chloride. The results of the cultures on solid media were identical with those obtained with the bouillon, the tubes containing phosphate giving a much more rapid and abundant growth than those containing chloride. The accompanying plates illustrate very well the results of these experiments. Plate II shows agar cultures of the same age made on sodium chloride and on acid potassium phosphate agar. All four of the tubes were inoculated with an equal amount of the same culture. This culture had been grown for a number of generations on a bouillon containing acid potassium phosphate but no sodium chloride. Plate III represents four tubes of the same lot of agar, all inoculated at the same time with the same amount of a culture which had been grown for more than eight years on a medium containing sodium chloride and to which no phosphate had been added.

It will be seen that although the culture which is shown in Plate III had been accustomed for many years to the sodium chloride broth, it grew much more vigorously in the first generation upon agar containing acid potassium phosphate. As a result of the above-described experiments, which were confirmed by subsequent work, we have discontinued entirely the addition of sodium chloride to bouillon and agar intended for the cultivation of tubercle bacilli, and have substituted for it an equal amount of potassium hydrogen phosphate. Bouillon of this character, which has been in use in the Biochemic Division for several years, and which has given uniformly satisfactory results, is prepared as follows: To 1 part of freshly chopped meat 2 parts of distilled water are added and the mixture is kept at a temperature of 45° to 58° C. for three hours. The broth is now strained,

boiled, and filtered; 1 per cent of Witte's peptone and one-half of 1 per cent of acid potassium phosphate are added. After these ingredients have been completely dissolved, sodium hydrate is added until the reaction is approximately 1 per cent acid, phenolphthalein being used as an indicator. The solution is now boiled for one hour and filtered. Seven per cent glycerine is added and the reaction, if affected by boiling, is again brought to the previous degree of acidity—that is, 1 per cent acid to phenolphthalein. The completed bouillon is now put in flasks and sterilized in the usual manner. Agar may be prepared from the above-described broth.

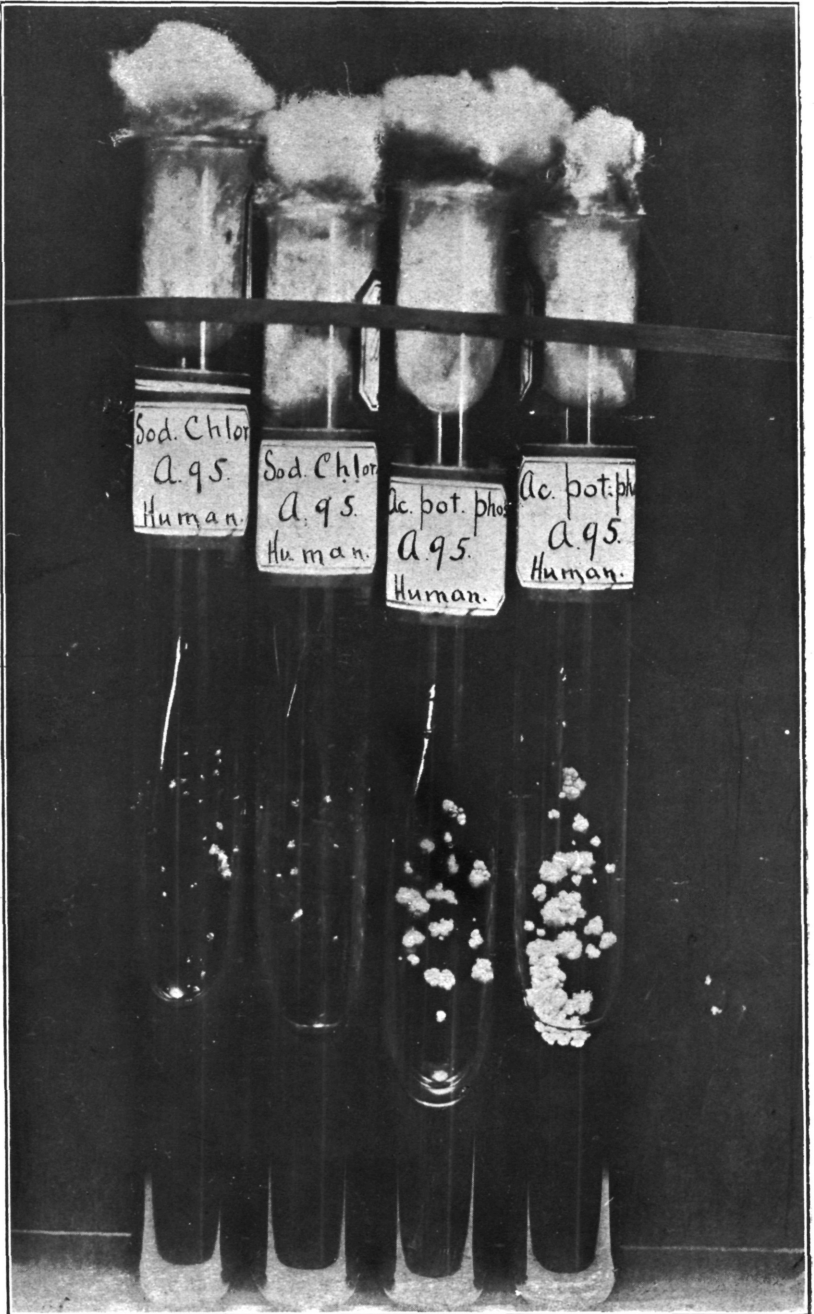
A few attempts have been made to cultivate tubercle bacilli directly from the tissues of guinea pigs upon agar containing acid potassium phosphate, but so far without marked success, the agar prepared in this way being apparently much less satisfactory for obtaining the first growth of tubercle bacilli from animals than hardened egg or dog's serum.

In 1894 Proskauer and Beck,<sup>a</sup> starting with Kühne's synthetic medium as a basis, tried various combinations of the inorganic salts, their object being to eliminate those which were of no use and to determine also what elements were absolutely essential for the growth of tubercle bacilli. The simplest medium upon which they were able to obtain a growth of the tubercle bacillus contained commercial ammonium carbonate, primary potassium phosphate, magnesium sulphate, and glycerine, dissolved in water. This medium was not the most favorable one for the growth of the tubercle bacillus, however, but the elements mentioned were found to be essential to its development. It will be seen that they did not find it necessary to add a chloride to the solution.

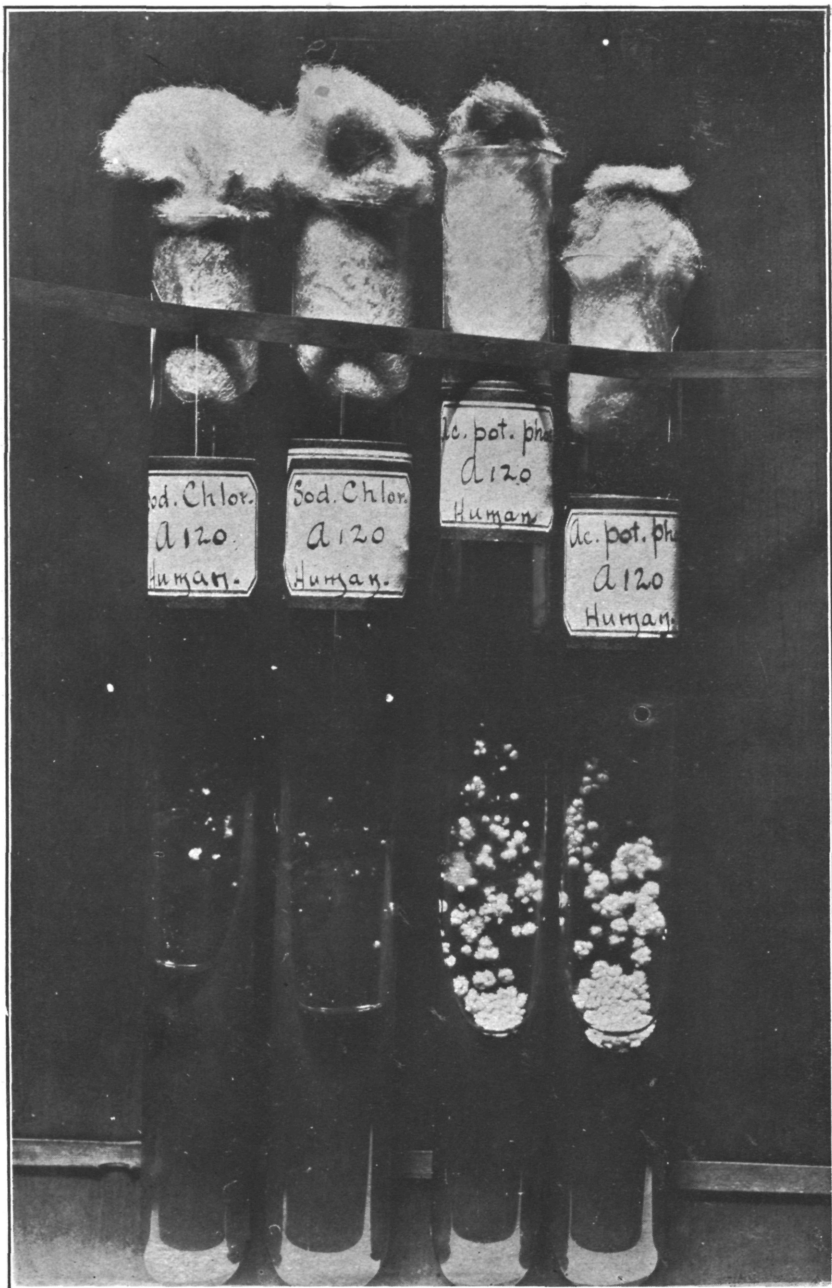
Inasmuch as all of the text-books on bacteriology recommend the use of the sodium chloride bouillon, glycerinized, for the cultivation of tubercle bacilli, it has seemed desirable to call the attention of workers in bacteriological lines to the medium which has been used in the Biochemic Laboratory for six years and which has proven itself in our hands far superior to that recommended by the text-books. We have been unable to note any change in the virulence of our cultures as a result of their growth upon the phosphate bouillon, and tuberculin prepared from such cultures has proven to be perfectly satisfactory.

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<sup>a</sup> Zeitschr. f. Hyg. u. Infektionskr., Leipz., 18 Bd., p. 128. 1894.



TUBERCULOSIS CULTURES ON SODIUM CHLORIDE AND PHOSPHATE AGAR AFTER HAVING GROWN FOR MANY GENERATIONS ON SODIUM CHLORIDE BOUILLON.



TUBERCULOSIS CULTURES ON SODIUM CHLORIDE AND PHOSPHATE AGAR AFTER HAVING GROWN FOR SEVERAL GENERATIONS ON POTASSIUM PHOSPHATE BOUILLON.